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A Novel Protein Encoded by the *InaD* Gene Regulates Recovery of Visual Transduction in Drosophila

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Summary

***InaD*^{p215} is a point mutation that affects photoreceptor function in Drosophila. To understand the molecular basis of the defect, we isolated the *InaD* gene and found it encodes a photoreceptor-specific polypeptide of 674 residues. Within its sequence are two repeats that share remarkable homology with a family of cytoskeleton-associated proteins that are involved in signal transduction. Patch-clamp recordings from isolated photoreceptor cells of *InaD*^{p215} show a slow deactivation of the light-induced current. This defective deactivation of *InaD* appears dependent on calcium influx; removal of extracellular calcium masks its abnormal phenotype. Moreover, *InaD* photoreceptors show increased sensitivity to dim light. We propose that InaD is involved in the negative feedback regulation of the light-activated signaling cascade in Drosophila photoreceptors.**

Introduction

Phototransduction is the process that uses the energy of light to generate an internal messenger, which subsequently leads to a transient change of membrane potential in photoreceptor cells. The mechanism of phototransduction in vertebrates has been subjected to many studies and is considered as one of the model systems for G protein–coupled signal transduction. In the visual cascade, light activates rhodopsin, which interacts with a heterotrimeric GTP-binding protein (transducin) to catalyze the exchange of GDP for GTP at the G_α subunit. The GTP-bound form of the transducin α subunit, in turn, activates a cyclic GMP (cGMP) phosphodiesterase that hydrolyzes cytosolic cGMP to GMP. The reduction of cGMP leads to the closure of cGMP-gated cation channels and hyperpolarization of photoreceptor cells (Stryer, 1986; Hurley, 1992; Lagnado and Baylor, 1992). Although the mechanism underlying visual activation is well studied, little is known about processes leading to recovery and adaptation of the cascade.

Drosophila renders itself as an ideal model system to study many unanswered questions concerning regulation of the visual cascade. The initial events of phototransduction in Drosophila, as well as other invertebrates, are similar to those in vertebrates. Indeed, genes encoding rhodopsins (O'Tousa et al., 1985; Zuker et al., 1985; Cowman et al., 1986; Montell et al., 1987; Zuker et al., 1987) and α (Lee et al., 1990) and β subunits (Yarfitz et al., 1991) of the eye-specific GTP-binding protein have been identified and characterized. However, following the activation of G proteins, the vertebrate and invertebrate cascades diverge. In invertebrates, GTP-bound G_α subunits appear to activate a phospholipase C (PLC; Yoshioka et al., 1984; Bloomquist et al., 1988), leading to hydrolysis of phospholipid to produce inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 releases calcium from intracellular stores (Berridge and Irvine, 1984, 1989; Ferris and Snyder, 1992; Henzi and MacDermott, 1992), whereas DAG is a potent activator of protein kinase C (Takai et al., 1979; Nishizuka 1986). To date, the identity of the intracellular messengers that open the light-activated channels in invertebrates and lead to depolarization, not to hyperpolarization as in vertebrates, have not been firmly identified. IP_3, calcium, and cGMP are candidates as mediators of visual excitation (Fein, 1986; Johnson et al., 1986; Devary et al., 1987; Baer and Saibil, 1988). The molecular entities of the light-activated channels also remain unknown, although recently it has been suggested that light-induced depolarization of photoreceptor cells in Drosophila is mediated by at least two functionally distinct classes of channels. The *transient receptor potential* (*trp*) mutation selectively abolishes the class of channels possessing high calcium permeability (Hardie and Minke, 1992).

We have taken advantage of available Drosophila visual mutants to isolate and characterize genes coding for novel polypeptides that may play a role in visual transduction. Knowledge about these mutations will provide insight not only into the molecular basis of visual transduction but also into the regulation of other G protein–coupled PLC-mediated signaling mechanisms.

In this paper we report the molecular and electrophysiological characterization of the *inactivation no afterpotential D* (*InaD*) mutation in Drosophila. The *ina* visual mutations, identified based on their abnormal electroretinograms (ERG) by Pak and colleagues, fall into five complementation groups, *inaA–inaE* (Pak, 1979). *InaD*^{p215} is the one and only allele that is a dominant mutation. Electrophysiological analysis of the mutant suggests that InaD is critically involved in the regulation and deactivation of the visual cascade, as *InaD*^{p215} shows a slow recovery of light-induced responses and altered light sensitivity. We have isolated the *InaD* gene by subtractive hybridization and carried out some molecular characterization of the putative *InaD* gene product to elucidate the role of InaD in visual cascade.

Results

InaD Is Dominant and Displays an Abnormal ERG

An ERG is the extracellular recording of the light-induced electrical activity in the compound eye. A commonly used ERG paradigm to look for mutants with defective vision is to stimulate white-eyed flies with intense light of various wavelengths. In particular, an intense blue light (λ480 nm), which corresponds to the activation wavelength of the ma-

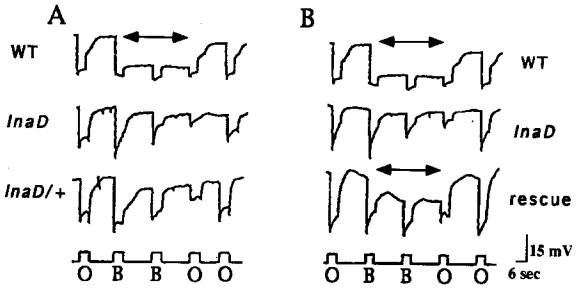

Figure 1. ERG Recordings of Wild-Type, *InaD*, and Rescue Flies

(A) *InaD* is a dominant visual mutation. Shown are ERG recordings of wild-type (WT), *InaD* homozygote (*InaD*), and heterozygote (*InaD*/+) flies, in response to intense light (duration = 6 s, as indicated in the bottom tracing). Wavelengths of light delivered are indicated below. A pulse of intense blue light triggers a PDA (double arrows) in wild-type, but not in *InaD* nor in *InaD*/+ flies. O, orange light (λ580 nm); B, blue light (λ480 nm).

(B) Restoration of wild-type physiology in $InaD^{p215}$ by expression of the putative *InaD* gene product. ERG recordings of wild-type (WT), *InaD* (*InaD*), and transgenic flies (rescue, or p*InaD*+; *InaD*) carrying two wild-type copies of *InaD* driven by the *hsp70* promoter in the mutant *InaD* genetic background. The expression of *InaD*+ in the transgenic flies was induced by heat shock. Heat shock transgenic flies display almost wild-type physiology, regaining partial PDA.

jor rhodopsin, will convert a substantial amount (>30%) of rhodopsin into metarhodopsin and bring about a prolonged depolarizing afterpotential (PDA) that persists even after the stimulus has been terminated. During a PDA, photoreceptor cells show a much reduced response to a subsequent pulse of blue light, which is thought to be due to inactivation of photoreceptor cells (when entering a complete PDA, the response to a subsequent pulse of blue light is absent). A PDA can be terminated by a pulse of orange light (λ580 nm), which photoconverts metarhodopsin to rhodopsin. In brief, the visual physiology of wild-type flies is characterized by the presence of a PDA and inactivation of photoreceptor cells by a pulse of blue light (Figure 1A).

In contrast, the response of *InaD* flies to blue light is characterized by the absence of a PDA. Despite not being able to maintain a PDA, the photoreceptors become inactivated and show a much reduced response to a second blue light stimulus (Figure 1A). Moreover, $InaD^{p215}$ is dominant (i.e., both heterozygotes and homozygotes display abnormal phenotypes). The $InaD^{p215}$ homozygotes exhibit a much more severe phenotype, as the rate of initial decay following the peak response is greater than that of heterozygotes (Figure 1A). The cytogenetic location of *InaD* has been mapped to 58F1–60F1 (Lindsley and Zimm, 1992).

Expression of the Putative *InaD* Gene Rescues the Abnormal Physiology in Mutants

To understand the role of the *InaD* gene product in the visual cascade, we isolated the *InaD* gene. We searched among those eye-specific genes that were identified by subtractive hybridization (Shieh et al., 1989) for genes having a similar chromosomal location as *InaD*. One of 40 genes we identified, p59B, was mapped to 59B1–4 in the polytene chromosome (data not shown), within the chromosomal location of the *InaD* locus. To establish whether or not the p59B is the *InaD* gene, we used genetic complementation to investigate whether overexpression of a wild-type copy of p59B was able to rescue the mutant phenotype. We introduced a full-length cDNA of p59B driven by a *hsp70* promoter into *InaD* flies by P element–mediated germline transformation (Rubin and Spradling, 1982). The expression of p59B cDNA was induced by heat shock treatment (37°C for 1 hr every 12 hr following emergence) in the transgenic flies. Transgenic flies displayed near wild-type physiology, regaining PDA and inactivation in three independent lines (Figure 1B), whereas heat shock treatment did not affect wild-type and *InaD* ERG phenotypes. The rescue is dependent on the copy number of the transgene; two rather than one copy of the wild-type *InaD* completely rescued the abnormal physiology of *InaD* heterozygotes (data not shown). However, heat shock–induced expression of the wild-type InaD protein did not completely rescue the phenotype in *InaD* homozygotes (Figure 1B). Flies reared at 25°C without heat shock displayed the mutant phenotype.

Characteristics of the *InaD* Gene Product

We obtained several cDNAs coding for *InaD* from a Drosophila head cDNA library and determined the nucleotide sequence of the longest cDNA for both strands. We searched for the open reading frame and obtained a 2022 bp open reading frame that coded for a polypeptide of 674 amino acid residues (Figure 2A). The first methionine at the nucleotide +75 in the sequence was assigned arbitrarily to be the initiator methionine, although the sequence (TCACAUG) flanking the second methionine (at amino acid 17) is perhaps better fitted as the translation start site (consensus sequence of the translation initiation sites in Drosophila, [C/A]AA[A/C]AUG; Cavener, 1987). A Kyte-Doolittle hydrophilicity plot of the predicted InaD protein sequence (Figure 2B) reveals no stretches of hydrophobic sequences of 20 or more residues in length, suggesting that InaD is not likely to be an integral membrane protein. Nor is there a putative signal peptide sequence in InaD that would implicate InaD as a secreted protein. InaD is extremely rich in both basic (15.4%) and acidic (14.1%) amino acid residues, with a predicted pI of 8.66. Most of these charged residues are distributed throughout the entire molecule, with the exception of a stretch of 14 amino acids (amino acids 460–473), which is made up exclusively of lysine and glutamic acid residues. Repeats consisting of GQ(M) are present between amino acids 142 and 158 (Figure 2A). Sequence analysis also reveals potential phosphorylation sites (Kennelly and Krebs, 1991) of cAMP- and cGMP-dependent protein kinases (one site), tyrosine kinases (one site), and protein kinases C (eight sites; Figure 2A), suggesting that InaD may be potentially regulated by these modifications. Also of considerable interest is the finding that two repeats of 40 amino acids of InaD share sequence similarity with repeats in a family of proteins that includes the Drosophila disc-large (dlg; Woods and Bryant, 1991), the rat post-synaptic density protein (PSD95; Cho et al., 1992), the vertebrate tight junction protein ZO-1 (Ito et al., 1993), and the human ros

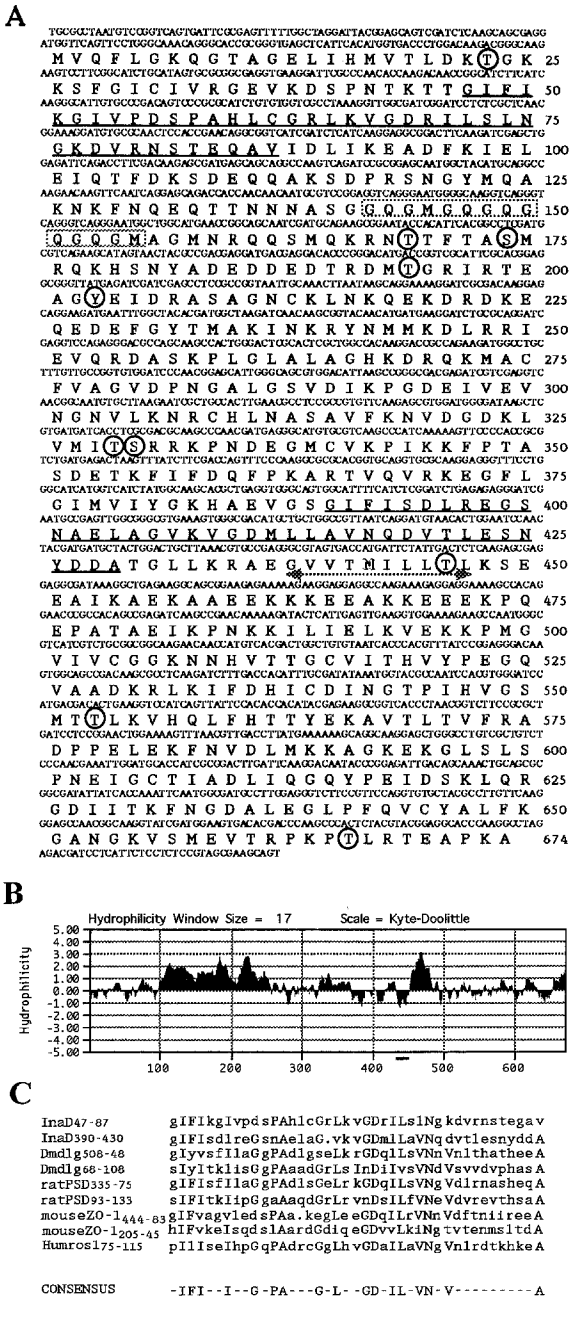

Figure 2. The *InaD* Sequence

(A) Shown is one of the longest obtained cDNAs of *InaD* and its predicted translational product. Amino acids are shown as single-letter codes, and numbering begins at the initiator methionine. Two repeats that share homology to dlg and PSD95 are underlined. Repeats containing GQ(M) are boxed. The point mutation in the *InaD*^{p215} is outlined (amino acid 442). This methionine is located within a hydrophobic stretch (double arrows) of the InaD protein and was mutated to a lysine in *InaD*^{p215}. Potential phosphorylation sites for the following kinases are circled: protein kinase C sites (at amino acids 23, 174, 194, 329, 330, 446, 553, and 666), cAMP/cGMP-dependent protein kinases (amino acid 169), and tyrosine kinases (amino acid 203).

(B) The Kyte-Doolittle hydrophilicity profile (window size, 17) of the predicted InaD protein. This analysis was performed using the MacVector program (IBI). InaD appears to be very hydrophilic and does not have readily apparent stretches of hydrophobic amino acid residues (< −1.5) that would correspond to predicted α-helical transmembrane domains. The hydrophobic domain where the mutation in *InaD*^{p215} was located is indicated as a bar.

(C) The sequence alignment of two repeats within InaD with Drosophila dlg, rat PSD95, mouse ZO-1, and human ros. The search was originally performed at NCBI using the BLAST network service. The consensus sequence was derived based on the frequency (more than 6 out of 9 repeats) that a residue is present in corresponding positions of different repeats.

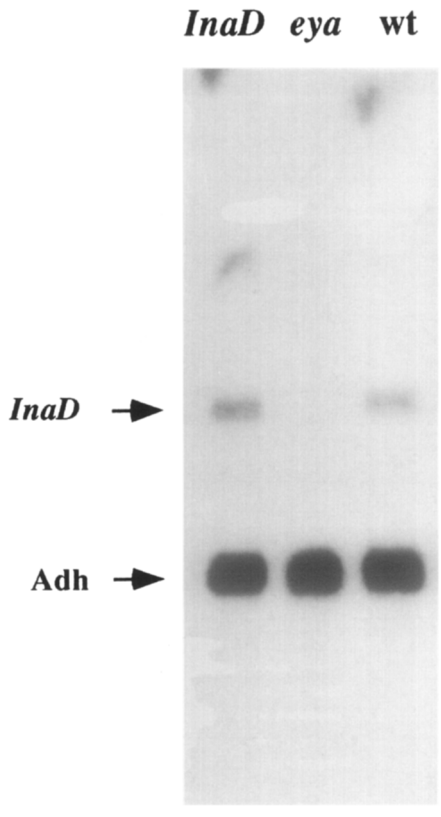

Figure 3. Northern Blot Analysis of *InaD*

Poly(A)$^+$ RNA from heads of the fly was size-fractionated on a 1% agarose/formaldehyde gel and transferred onto a filter membrane. Radiolabeled *InaD* probe was used to examine the expression of the *InaD* transcript. A Drosophila alcohol dehydrogenase (Adh) probe was used as a control. The *InaD* transcript is about 2 kb in size and is expressed in heads of wild-type and *InaD*^{p215} flies, but absent in *eya* mutants. wt, wild-type heads; *eya*, heads of *eya*; *InaD*, heads of *InaD*^{p215}.

protein (Birchmeier et al., 1990; Figure 2C). The identity among the first repeat of InaD and others ranges from 32% to 51%.

InaD mRNA Expression

We carried out Northern blot analysis to investigate the expression of *InaD* in heads of wild-type and mutant flies. As shown in Figure 3, we detected the presence of a 2 kb *InaD* transcript in wild type, which is absent in the *eyes absent* (*eya*) mutant that lacks compound eyes (Sved, 1986). These results are consistent with the findings that the isolation of *InaD* is based on its eye-specific expres-

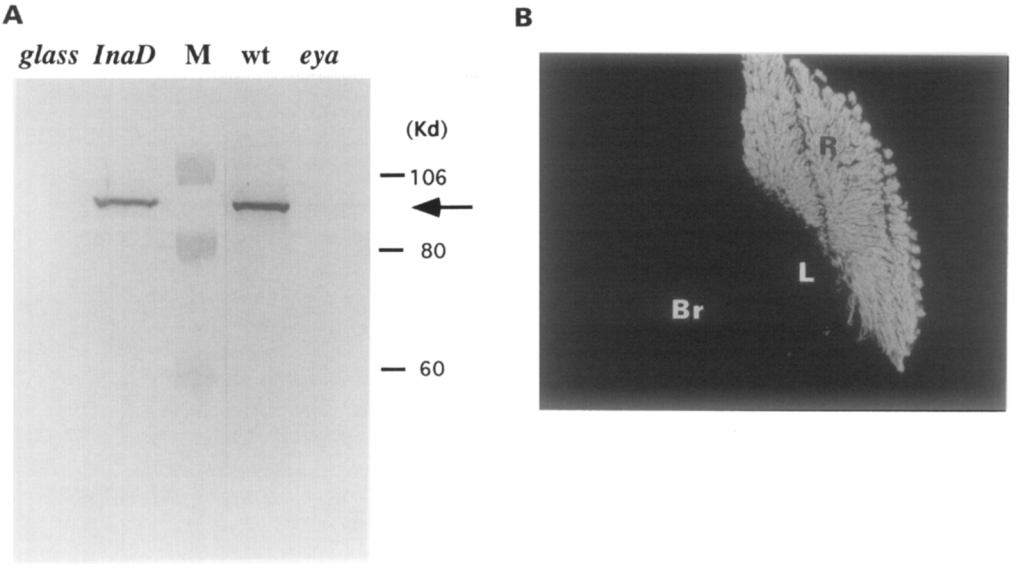

Figure 4. Expression of the *InaD* Gene Product

(A) Western blot analysis of InaD. Homogenates prepared from wild-type and mutant flies were size fractionated in 7.5% SDS–PAGE and transferred onto a nitrocellulose filter. Subsequently, the filter was probed with anti-InaD antisera. Anti-InaD antibodies recognized a protein of 90 kDa in size, which is present in heads of wild-type (wt) and *InaD* (*InaD*). The protein is drastically reduced in heads lacking compound eyes (*eya*), and absent in fly heads missing photoreceptor cells (*glass*). Molecular weight standards are indicated.

(B) Immunofluorescent localization of InaD. Shown is the fluorescent view of a 10 μm frozen section of a wild-type fly head that was stained with anti-InaD antisera, followed by rhodamine-conjugated secondary antibodies. InaD is expressed exclusively in the retina of the compound eye. R, retina; L, lamina; Br, brain.

sion. Interestingly, the *InaD* mRNA is also present in *InaD* mutant, with size and abundance comparable to that of wild-type flies (Figure 3).

Expression of the *InaD* Gene Product

We obtained polyclonal antibodies by immunizing rats with a bacterial fusion protein of InaD corresponding to the polypeptide spanning from amino acids 280 to 550 of InaD. The antisera were used in Western blot analysis to examine the expression of the InaD protein. Anti-InaD antibodies recognized a protein of about 90 kDa in extracts prepared from heads of wild-type flies. This putative InaD protein is drastically reduced in heads of *eya* and absent in heads of *glass*, which lack photoreceptor cells (Figure 4A). The *InaD* gene product is also present in $InaD^{p215}$ heads (Figure 4A). Thus, $InaD^{p215}$ mutation may not be due to the absence of the InaD protein, but to a change of activity of the *InaD* gene product in the mutant.

The InaD protein extracted from fly heads exhibits an apparent molecular weight of 90 kDa, which is larger than its estimated size of 80 kDa. To examine whether the increase in size is due to phosphorylation, we compared the molecular weights of InaD treated with alkaline phosphatase with an untreated one. We found that phosphatase treatment did not affect the electrophoretic mobility of InaD (data not shown), suggesting that the discrepancy in apparent and estimated molecular weights of InaD is not due to phosphorylation.

InaD Is Found Exclusively in Photoreceptor Cells

To examine the subcellular distribution of InaD in the compound eye of flies, we carried out indirect immunofluorescence in frozen sections of fly heads. Anti-InaD antisera recognized InaD present exclusively in the retina of the eye, confirming that *InaD* is expressed in photoreceptor cells (Figure 4B). Similarly, the antisera also stained three ocelli, simple eyes located at the apex of head (data not shown). This finding is consistent with the Western blot analysis of *eya* heads that showed detectable InaD protein likely to be contributed by the ocelli. In ultra thin sections of photoreceptor cells, it appears that most of InaD is closely associated with rhabdomeres (Colley and B.-H. S., unpublished data), which are visual organelles consisting of densely packed microvilli.

Sequencing Analysis of the Mutant Allele Reveals a Point Mutation in $InaD^{p215}$

$InaD^{p215}$ flies express the *InaD* gene product at a level similar to that of wild-type flies. To determine the molecular basis of the mutation, we isolated and sequenced the mutant allele. We designed oligonucleotide primers to amplify the coding region of $InaD^{p215}$ gene (about 3 kb in size) by polymerase chain reaction (PCR), using total genomic DNA from the mutant as the template. Subsequently, the amplified genomic DNA was subcloned and subjected to nucleotide sequencing. To account for mutations that may have been introduced during the amplification by Taq DNA polymerase, we sequenced DNA from two independent PCR reactions and obtained consistent results. We found a missense mutation that converts a methionine (ATG) to a lysine (AAG) at the amino acid residue 442 in the mutant gene (see Figure 2A). This methionine is located in one of the hydrophobic regions of the InaD protein (see Figure

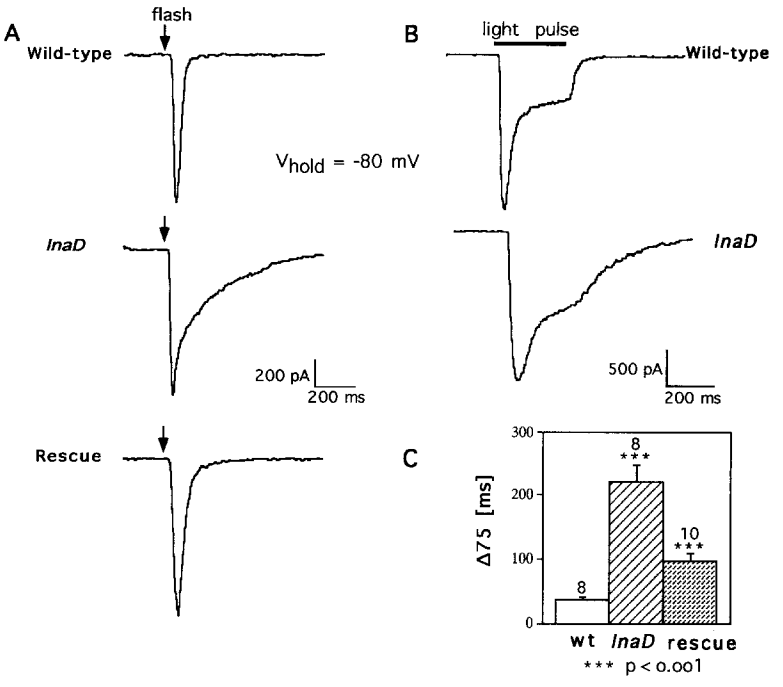

Figure 5. *InaD* Photoreceptor Cells Display Slow Recovery of Light-Induced Currents, Which Is Rescued by Expression of the Wild-Type *InaD* cDNA

Light-induced currents of wild-type and *InaD* photoreceptor cells in response to either a flash (A) or pulse (B) of light, recorded in whole-cell patch-clamp conditions at a holding potential of −80 mV. *InaD* photoreceptors exhibit slower deactivation of light-induced responses to either a flash (A) or a pulse (B) of light. Heat-shocked transgenic flies (rescue, p*InaD*+; $InaD^{p215}$) carrying two copies of the wild-type *InaD* driven by the *hsp70* promoter in the $InaD^{p215}$ genetic background display nearly wild-type deactivation kinetics, as shown in (A). In light pulse-induced currents (B), *InaD* cells desensitize to a lesser extent. The rate of deactivation was quantified by measuring the time to 75% recovery (Δ75) of the peak amplitude in response to a flash of light (C). Overexpression of wild-type *InaD* rescues the abnormal deactivation in the mutant. Numbers of cells recorded are indicated.

2B), and therefore, the point mutation results in a change of hydrophobicity of the domain.

InaD Photoreceptor Cells Display Abnormal Deactivation of Light-Induced Currents

Data from ERG measurements suggest that InaD plays an important role in normal functioning and regulation of the visual cascade. To characterize this role more specifically, we recorded from single photoreceptor cells in the whole-cell patch-clamp configuration (Hardie et al., 1991; Ranganathan et al., 1991). Cells were voltage clamped at −80 mV and stimulated with attenuated light (λ520 nm).

Figure 5A shows light-induced currents recorded from photoreceptors of wild-type, *InaD*, and heat-shocked transgenic flies carrying the putative *InaD* cDNA in the $InaD^{p215}$ background (rescue) in response to a flash of light of 10 ms duration and a relative intensity of log unit −1. A flash of light triggers a transient inward current in wild-type photoreceptors. Light-induced currents from *InaD* cells display activation kinetics similar to those of wild-type cells, yet display a striking defect in their deactivation kinetics; that is, the rate of recovery to baseline following the peak response is significantly decreased (Figure 5A). Heat shock–induced expression of the wild-type *InaD*

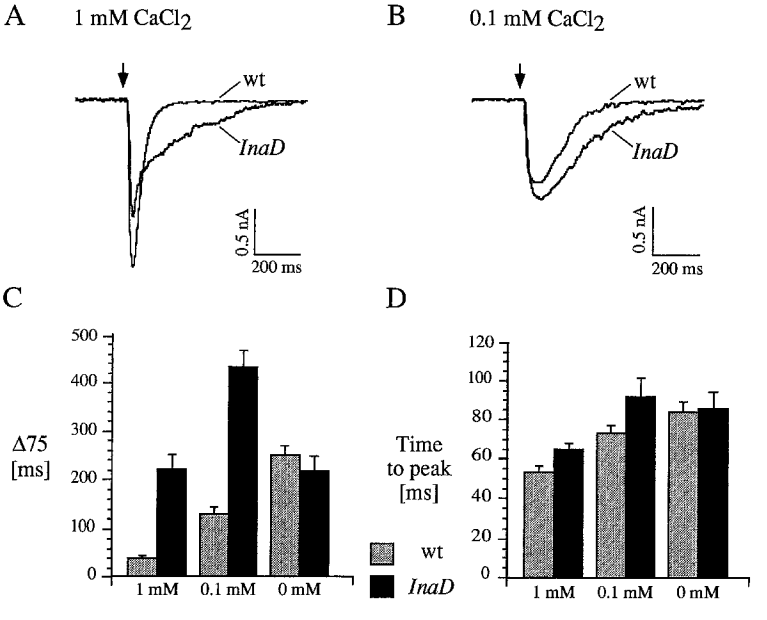

Figure 6. The Defect of *InaD* Depends on the Concentration of Extracellular Calcium

Extracellular calcium modifies the kinetics of light-induced currents. Superimposed light-induced currents of wild-type and *InaD* photoreceptor cells in bath solutions containing either 1 mM (A) or 0.1 mM calcium (B) at a holding potential of −80 mV. The kinetics of inward currents in the presence of varying concentration of calcium were analyzed as time to 75% recovery, Δ75 (C), and time to peak (D). Both bar graphs show data from wild-type (black bars) and *InaD* mutant (grey bars). In bath solution containing calcium, *InaD* photoreceptors exhibit slower deactivation that is not apparent when photoreceptor cells were analyzed in the absence of extracellular calcium. On the average, the time to peak is not significantly increased in the mutant (D).

gene in the mutant rescues this defect, thus demonstrating that the defective deactivation is indeed due to the mutation of *InaD*. To analyze the currents, we measured latency, time to peak, and time to repolarization by 75% of the peak current (Δ75). Figure 5C shows the Δ75 values for averaged responses (mean ± SEM) of 8 wild-type (37 ± 5 ms), 8 *InaD* (220 ± 27 ms), and 10 rescue cells (96 ± 12 ms). This deactivation defect in the mutant is rescued by the heat shock–induced expression of the wild-type *InaD* gene product. The increased recovery time of the rescue cells is due to the heat shock treatment, as wild-type and *InaD* flies treated similarly also display slower kinetics (see time constants [τ] below). Most of the recordings were conducted in the presence of CsCl. Since extracellular cesium blocks the electrogenic sodium–calcium exchanger, we confirmed all of our results using a cesium-free bath solution (Ringer's with 1.5 mM $CaCl_2$; see Hardie et al., 1993) at a holding potential of −40 mV. Furthermore, we approximated a single exponential fit to the last 20% of the normalized light-induced current and found that the resulting τ values show similar differences as the Δ75 values (τ for wild type, 24 ± 10 ms [n = 4]; for *InaD*, 149 ± 16 ms [n = 4]; for heat shock rescue, 73 ± 6 ms [n = 2]; for wild type [with heat shock], 71 ± 18 ms [n = 3]; for *InaD* [with heat shock], 430 ± 85 ms [n = 4]). Latency and time to peak are only marginally affected in the mutant, suggesting that the time course of activation is not directly affected (latency at 1.5 mM $[Ca^{2+}]_o$: wild type, 28.5 ± 1.9 ms, *InaD*, 29 ± 1.4 ms; time to peak: wild type, 75.5 ± 12 ms, *InaD*, 85 ± 4.2 ms; see Figure 6D).

In response to light pulses of 300 ms duration (Figure 5B), both wild-type and *InaD* mutants show a complex response with a fast initial desensitizing component during the light pulse (adaptation). However, following the termination of light, the deactivation of light-induced currents in *InaD* photoreceptors is prolonged, as revealed by Δ50, which is the time to repolarization by 50% of the steady-state current, 183 ± 54 ms (n = 12) compared with 53 ± 4 ms (n = 7) in wild type. The appearance of a multiphasic response by a pulse of light is a manifestation of adaptation and is dependent on the influx of calcium; light pulse–triggered currents recorded in nominally zero calcium do not exhibit desensitization that leads to a lower steady-state response (Ranganathan et al., 1991). The finding that *InaD* shows desensitization to a pulse of light suggests that light adaptation is not abolished. However, *InaD* cells display a lesser degree of desensitizaton than wild type. If the amplitude of the steady-state current is expressed as percent of initial peak amplitude to represent the degree of desensitization, wild type desensitizes to an average of 35%, whereas *InaD* only desensitizes to an average of 56% (see also Figure 5B; for wild type, n = 7; for *InaD*, n = 12; $p < .01$). These findings suggest that regulation of the light response through a calcium-mediated process is affected in the mutant.

The Abnormal Deactivation Kinetics in *InaD* Are Dependent on Extracellular Calcium

Activation and deactivation mechanisms of the photoreceptor response in Drosophila are strongly influenced by the amount of calcium entering the cell from the extracellular space (Lisman and Brown, 1972; Ranganathan et al., 1991; Hardie et al., 1993). To investigate the role of extracellular calcium on the *InaD* deactivation phenotype, we recorded from photoreceptors in the presence of 1 mM (Figure 6A), 0.1 mM (Figure 6B), and nominally zero $CaCl_2$ bath solutions. Figure 6C shows the resulting Δ75 values (in 1 mM $CaCl_2$: wild type, 37 ± 5 ms [n = 8], *InaD*, 220 ± 27 ms [n = 8]; in 0.1 mM $CaCl_2$: wild type, 128 ± 15 ms [n = 4], *InaD*, 430 ± 35 ms [n = 9]; in 0 mM $CaCl_2$: wild type, 249 ± 19 ms [n = 2], *InaD*, 215 ± 31 ms [n = 4]). In zero external calcium, the deactivation kinetics of wild type and *InaD* are not significantly different. This can be expected if *InaD* acts at a calcium-mediated step of deactivation; in zero external calcium this step would not be activated in either wild-type or mutant photoreceptors. The times to peak (Figure 6D) increase with decreasing calcium and are always slightly longer in *InaD* cells (in 1 mM $CaCl_2$: wild type, 53 ± 3 ms [n = 8], *InaD*, 65 ± 3 ms [n = 8]; in 0.1 mM $CaCl_2$: wild type, 73.3 ± 4 ms [n = 4], *InaD*, 92 ± 9 ms [n = 9]; in 0 mM $CaCl_2$: wild type, 84 ± 5 ms [n = 2], *InaD*, 87 ± 8 ms [n = 4]), which could be accounted for by assuming an increased level of dark adaptation.

Taken together, these results suggest that the abnormal deactivation in *InaD* is dependent on the rapid light-induced rise in intracellular calcium concentration through influx of extracellular calcium.

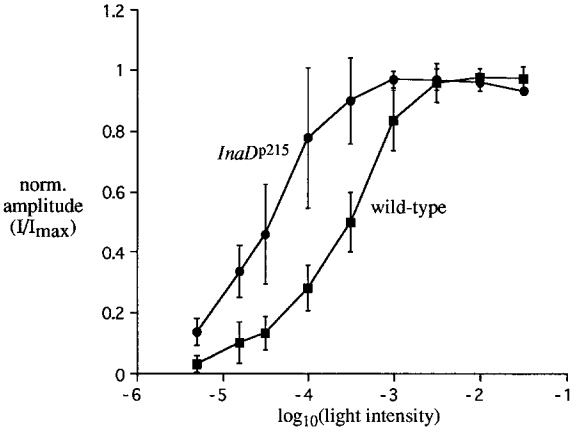

Figure 7. *InaD* Photoreceptors Display Increased Sensitivity to Light

Shown are intensity-response plots of wild-type (closed squares) and *InaD* (closed circles) photoreceptors in response to 10 ms flashes of λ520 nm light at a holding potential of −40 mV. After dissociation, photoreceptor cells were dark adapted and then stimulated with light of increasing intensity. The amplitudes of light-induced inward current were normalized to the maximal response obtained. *InaD* cells are more sensitive to dim light stimulation as the intensity-response curve is shifted to the left.

Mutation of the InaD Protein Increases Sensitivity toward Dim Light Stimuli

To investigate whether the *InaD* mutation affects the sensitivity of the response, we measured the responses of dark-

adapted photoreceptor cells to 10 ms stimuli of increasing light intensity (λ520 nm) in physiological bath solution. Figure 7 shows the resulting intensity response plots of five wild-type and five *InaD* cells; currents were normalized to the maximal amplitude of each cell and plotted against the log of light intensity. *InaD* photoreceptors are significantly more sensitive towards dim light stimuli, and the light intensity needed to reach half saturation of the light–dose response is 10-fold less than in wild-type flies. The mutation thus seems to increase the state of dark adaptation.

If the same sequences of increasing light stimuli are given to the same cells with an additional constant dim background light illuminating the recording chamber, the intensity response curves of *InaD* shift toward higher light intensities (light adaptation; data not shown). Whether the extent of light adaptation is different from wild type is not easily determined in the patch-clamp configuration, as cells do not survive multiple rigorous stimulus protocols.

Discussion

In this paper, we report the molecular characterization of a novel polypeptide, InaD, which appears to be involved in Drosophila visual transduction. *InaD* was isolated by a subtractive hybridization protocol that was designed to identify genes preferentially expressed in the compound eyes of Drosophila. The *InaD* gene product is a protein of 674 amino acid residues that is found exclusively in photoreceptor cells. When compared with known sequences in the database, we found that InaD shares limited homology with a class of proteins, including the Drosophila dlg, the rat PSD95, vertebrate ZO-1, and the human ros, within two repeats of 40 amino acids. These two repeats are within the GLGF repeats (1 and 3) reported previously by Cho et al. (1992) in the PSD95 sequence. The Drosophila dlg, PSD95, and ZO-1 belong to a family of proteins that are characterized by containing three distinct domains: three GLGF repeats in the N terminus, an SH3 domain, and a region similar to the yeast guanylate kinase. The *dlg* gene product is a cytoskeleton-associated protein consisting of 960 amino acid residues that was shown to be localized in the apical belt of the lateral membrane of epithelial cells. Mutation of *dlg* leads to abnormal embryonic development and neoplastic overgrowth of the imaginal discs. It was suggested that dlg acts as a tumor suppressor to regulate cell proliferation and differentiation in the fly (Woods and Bryant, 1991). The rat PSD95 protein is concentrated in the postsynaptic density fraction, which is composed of submembranous cytoskeleton particularly enriched beneath the postsynaptic membrane in the CNS (Cho et al., 1992). A second dlg homolog, the vertebrate ZO-1, is a protein present in tight junction (zonula occludentes) of epithelial cells (Ito et al., 1993). ZO-1 colocalizes with cadherins on the cytoplasmic face of cell–cell contact sites in epithelial and nonepithelial cells, and it may be involved in intercellular transport/signaling. The human *ros* is a mammalian *sevenless* homolog (Birchmeier et al., 1990). The *sevenless* gene product is a receptor tyrosine kinase that is involved in signaling and cell fate determination in Drosophila (Hafen et al., 1987). The expression of *ros* has been shown to be restricted during the induction and proliferation of epithelium during organogenesis of the kidney and intestine in mice (Sonnenberg et al., 1991; Tessarollo et al., 1992). These proteins sharing similarity with InaD appear to participate in quite diversified signal transduction processes. Although the function of these repeats is not known, perhaps they are involved in protein–protein interaction or some mechanisms common to signaling processes.

Electrophysiological analysis by both ERG and patch-clamp recordings reveals a distinctive phenotype and gives rise to several possible models of the role of InaD in Drosophila phototransduction. In ERG recordings, *InaD* displays the inactivation-no-afterpotential phenotype expected of the *ina* complementation group mutants (Pak, 1979), which is the inability to sustain a PDA after stimulation with strong blue light, as well as a reduced response to subsequent blue stimuli. Whole-cell patch-clamp recordings of *InaD* photoreceptor cells reveal the abnormal visual response at the cellular level. Under physiological conditions, *InaD* cells display a defect in deactivation, which appears rather late in the decay of the light-induced current (during the last 20%–30% of the decay) and is dependent on the influx of calcium. It has been shown that the influx of extracellular calcium is crucial for negative feedback regulation of the cascade (Lisman and Brown, 1972; Ranganathan et al., 1991; Hardie et al., 1993). *InaD* photoreceptors behave as though their negative feedback mechanisms have been reduced; they show an increased sensitivity towards dim light stimuli, display a late deactivation defect, and show increased levels of steady-state current during prolonged stimuli. The above phenotypes can be explained by assuming a certain basal level of negative feedback even in the dark. A defect thus might lead to increased production of a normally tightly regulated second messenger, thereby increasing sensitivity and prolonging the response. How could this defect in negative regulation lead to such a drastic ERG phenotype as the inability to sustain the blue light–activated PDA? In situations of very high and constant demand of the messenger (PDA), the lack of regulated supply might lead to depletion of the messenger pool and thus the inability to maintain a response. A lack of feedback regulation that leads to the exhaustion of an excitatory process has been proposed to explain the visual phenotypes in *inaC* (Smith et al., 1991; Hardie et al., 1993) and *trp* (Hardie and Minke, 1992) mutants.

The defect in *InaD* is less pronounced and occurs later than the deactivation defect seen in *inaC* mutants. Both *inaC* and *InaD* belong to the same class of visual mutations and display patch-clamp and ERG phenotypes that show different degrees of a related phenotype. Both InaD and inaC could act to modulate PLC, which is likely to be a potent regulator of photoreceptor gain. A defect in negative regulation of phosphatidylinositol-4,5-bisphosphate (PIP_2) hydrolysis could lead to an increased amount of IP_3 and DAG in low demand situations and result in increased sensitivity (i.e., higher gain in dim light conditions). When stimulated by intense light (as in the ERG), prolonged activation would result in depletion of phospholipid substrate

and, consequently, inactivation of photoreceptor cells to further stimulation. Since there are potential protein kinase C phosphorylation sites within the InaD sequence, it is possible that the *inaC* gene product that encodes the eye-specific isoform of protein kinase C (Smith et al., 1991) may regulate InaD by phosphorylation. This could explain the calcium dependency of the *InaD* phenotype and would place InaD functionally downstream of inaC. The same line of reasoning could apply if InaD acts as a negative regulator on the enzymes that are involved in PIP_2 synthesis and is consistent with the findings that PIP_2 is a minor component in the membrane and its synthesis is tightly regulated (Thomas et al., 1993).

The abnormal visual physiology observed in $InaD^{p215}$ is caused by a point mutation that converts the methionine at 442 residue into a lysine. This mutation does not alter the steady-state level of the *InaD* gene product, since it is also present in the mutant fly. However, it results in a change of the activity of the wild-type *InaD* gene product and leads to the defective physiology. We suggest that the domain around amino acid 442 in the *InaD* gene product is essential for wild-type InaD activity. Because this domain appears to be the most hydrophobic region within InaD, the point mutation may lead to a change of hydrophobicity and render a loss of function in the domain. *InaD* is a dominant mutation, as heterozygotes show abnormal phenotypes in ERG recordings. These data suggest either that two copies of wild-type product may be required for a functional visual cascade or, alternatively, that mutant InaD protein may inhibit the function of the wild-type protein. Perhaps InaD forms a multimer with itself or other signal transduction molecules. The mutant form of the InaD protein could interfere with the ability of wild-type protein to form functional complexes. Investigations to distinguish whether the mutant phenotype is due to haploinsufficiency of the locus or to the production of an inhibitory form of the InaD protein will be carried out once deficiencies of *InaD* are available.

In summary, we identified a novel protein that appears to regulate the deactivation of visual transduction in Drosophila. Further biochemical and electrophysiological characterizations of *InaD* are in progress to elucidate the role of InaD in visual cascade. Because of the availability of genetic manipulation in Drosophila, the knowledge gained about InaD will shed light on the function of other vertebrate homologs involved in diverse signaling processes.

Experimental Procedures

Molecular Biology Methodologies

cDNA Library Screening

The head cDNA library was plated and filter replicas of the library were probed with radioactively labeled *InaD* gene (random priming kit, Amersham) in a hybridization solution containing 5 × SSCP, 0.5% SDS, 10 mM EDTA, and 5 × Denhardt's at 65°C for 16 hr. Following hybridization, filters were washed with a buffer containing 0.1 × SSC and 0.1% SDS at 65°C for 2 hr (Sambrook et al., 1989). The positive plaques were identified by autoradiography and purified. The cDNA insert was isolated as an EcoRI fragment and subcloned into the Bluescript KS vector (Stratagene) for sequence analysis.

Sequencing

The nucleotide sequence was determined by the dideoxy chain termination method (Sanger et al., 1977), using a Sequenase kit (US Biochemicals). The coding region of the longest cDNA was sequenced from both strands using templates generated by nested deletions and by subcloning of overlapping restriction fragments.

Isolation of RNA and Northern Blot Analysis

Mass isolation of heads and bodies of the fly was carried out, first by freezing flies in liquid nitrogen and mechanically dislocating heads by vortexing frozen flies in a tube. The heads and bodies of the fly were then separated by sieving over stainless steel sieves that were chilled at −80°C.

Total RNA of the fly was isolated by homogenization of tissues using a polytron (Brinkmann) in a buffer containing 0.2 M NaCl, 10 mM Tris (pH 8.0), 1 mM EDTA, 1% SDS, and an equal volume of phenol. The homogenate was extracted with equal volume of chloroform, and the aqueous phase of the mixture was recovered by centrifugation. Extraction with phenol/chloroform was repeated twice, or until there were no more visible proteinous materials in the interface. Total nucleic acids were precipitated by ethanol and further purified by affinity chromatography over oligo(dT) 12–18 cellulose (Collaborative Research) to obtain mRNA.

Poly(A)$^+$ mRNA was fractionated on a 1% agarose/formaldehyde gel, blotted onto a nitrocellulose membrane, and probed with radiolabeled probes under standard hybridization condition. An RNA ladder (BRL) was used to provide molecular weight standards.

Isolation of Total Genomic DNA

Total genomic DNA was isolated by gently homogenizing flies in a homogenization buffer (100 mM NaCl, 100 mM Tris [pH 7.6], 100 mM EDTA, 0.5% SDS). The homogenate was incubated at 65°C for 30 min, and one-sixth volume of 8 M potassium acetate was added. The mixture was kept on ice for 20 min, and the supernatant was recovered following centrifugation. RNase was added to hydrolyze RNA, and the mixture was extracted twice with phenol/chloroform. Genomic DNA was precipitated by ethanol and quantified spectrophotometrically.

PCR

PCR was used to isolate the mutant allele of the *InaD* gene from the total genomic DNA of the *InaD* flies. Two primers (AAAGGTACCATGGTTCAGTTCCTGGGC and ATCGAATTCTGCACTGCTTCGCTACCG) with linkers (KpnI and EcoRI) corresponding to the 5′ and the complementary stand of the 3′ sequences were used to prime the synthesis of the *InaD* gene. This amplified DNA fragment was treated with restriction enzymes, was subcloned, and nucleotide sequencing was performed. The experimental conditions for PCR (30 cycles) were as follows: denaturation at 94°C for 1 min, annealing at 42°C for 1 min, followed by extension at 72°C for 10 min. The reaction mixture contained 250 ng of each primer, 2 μg of total genomic DNA as the template, 0.2 mM dNTP, and 2.5 U Taq DNA polymerase (Promega) in a buffer containing 10 mM Tris (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$.

Biochemical Techniques

Overexpression of Bacterial Fusion Protein/Polyclonal Antisera Production

A BamHI fragment of the *InaD* cDNA (corresponding to the coding region from amino acids 280 to 550) was subcloned into pET3b and introduced into Escherichia coli HMS174. The expression of the fusion protein was controlled by T7 RNA polymerase, which was provided by infecting bacteria with CE6 (Rosenberg et al., 1987; Studier and Moffatt, 1986). Following the addition of CE6 with a multiplicity of infection of 10, bacteria were allowed to grow at 37°C for an additional 3 hr. Bacterial cultures were collected, and the expression of fusion protein was analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). To isolate fusion protein, total bacterial proteins were subjected to SDS–PAGE, followed by staining with Coomassie blue without fixing of the gel. The band of proteins corresponding to the desired fusion protein was excised, electroeluted, dialyzed, and used as the antigen to immunize rats according to published procedures (Harlow and Lane, 1988).

Western Blot Analysis

Fly heads were dissected and homogenized in 2 × SDS–PAGE loading buffer by sonication. Extracts prepared were fractionated by SDS–PAGE (7.5%), and proteins were electrophoretically transferred onto a nitrocellulose filter (Schleicher and Schuell). To detect the expression of InaD, the filter was probed with rat antisera raised against InaD,

and the immunocomplexes were visualized by enzymatic reactions following the incubation of alkaline phosphatase–conjugated secondary antibodies.

Physiological Assays

Electroretinogram Recordings

Flies were anesthetized by CO_2 and mounted on slides to immobilize them. Glass electrodes were filled with standard saline (0.7% NaCl). To record the visual response, one glass electrode was placed on the surface of the compound eye and another in the thorax as a reference. Light stimulation was by means of a Xenon light beam passed through a high intensity grating monochromator. Intense blue light (λ480 nm) was used to activate R1–R6 photoreceptor cells, and the duration of light stimulation was set at 6 s to trigger a PDA. Unfiltered light intensity was 1.8×10^{-3} mW/cm^2 at sample level. All recordings were performed in a dark room in dim red light. Signals were amplified by means of a WPI Dam 60 preamplifier and digitized on a 1 MHz A/D board. Under standard conditions, the average response of ERG recordings was about 15 mV.

Whole-Cell Patch-Clamp Recording

The patch-clamp recording of dissociated photoreceptor cells was carried out as described (Hardie et al., 1991; Ranganathan et al., 1991). In brief, heads from stage p15 pupae were dissected, and the compound eyes were mechanically dissociated under a dissection microscope in a calcium-free, magnesium-free CsCl solution and transferred to the bath solution. Bath solutions contained either 124 mM CsCl, 10 mM HEPES, 32 mM sucrose, and $CaCl_2$ concentrations ranging from 0.1 mM to 1 mM (pH 7.15) or else contained 120 mM NaCl, 5 mM KCl, 8 mM $MgSO_4$, 5 mM proline, 10 mM HEPES, 25 mM sucrose, and 0.5–1.5 mM $CaCl_2$ (pH 7.15). The patch pipette was filled with 124 mM CsCl, 10 mM HEPES, 1 mM EGTA, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$ (or $MgSO_4$) (pH 7.15).

Recordings were made from photoreceptor cells of whole-cell capacitance over 30–35 pF. Only cells with seal resistance of >1 GΩ were used for whole-cell recordings, and we compensated for most series resistance errors (>80%). The cells were clamped at holding potentials of either −80 mV or −40 mV. Signals were amplified with an Axopatch 1-D patch-clamp amplifier (Axon), and the data were analyzed using pClamp 5.51 software (Axon Instruments, Foster City, CA). Photoreceptors were stimulated with light from a 75Ω Xenon arc lamp that was filtered through a 520 ± 10 nm interference filter and attenuated by various neutral density filters. In flies subjected to heat shocks, recordings were made between 1 and 5 hr after the last heat shock.

Other Procedures

Indirect Immunofluorescence

Fly heads were dissected, frozen in OCT compound (Miles, Inc.), and 10 μm sections were prepared by cryostat (Reichart-Yung). Tissue sections were fixed with 4% paraformaldehyde/1 × phosphate-buffered solution and incubated with rat anti-InaD antibodies for 1 hr at room temperature, followed by goat anti-rat IgG conjugated with rhodamine (Jackson ImmunoResearch Laboratories). The localization of InaD was then examined in a fluorescence microscope. Preimmune serum was used as a negative control.

Maintenance and Crosses of Fly Stocks

Fly stocks were maintained at 24°C in a 12 hr dark/12 hr light cycle. Crosses were carried out using standard techniques (Ashburner, 1989). *InaD*^{p215} was obtained from Dr. Pak (Purdue University).

P Element-Mediated Germline Transformation

We made a construct by ligating a full-length cDNA of *InaD* to the Drosophila *hsp70* promoter in a P element vector containing a neomycin phosphotransferase gene as a selection marker for injection to generate transgenic flies. Experimentally, embryos at appropriate stages were collected, dechorionated, and injected with the DNA construct along with a helper DNA that encoded the transposase. Following injection, the surviving adults were mated with a balancer stock, and offspring of this cross were selected for neomycin resistance by G418 (500 mg/liter). Each transgenic line was made homozygous, and the chromosome in which DNA was integrated was determined by standard genetic crosses.

In Situ Chromosomal Mapping

Polytene chromosome squashes (Canton S strain) were prepared as described (Zuker et al., 1985). Hybridization of biotinylated DNA probes was carried out accordingly (Smith et al., 1990). In brief, DNA was labeled by nick translation using Bio-16 dUTP (Enzo Biochemicals), and hybrids were detected by using a Detek I-HRP detection kit (Enzo Biochemicals).

Statistical Analysis

Data were entered into Statview II and analyzed using unpaired t test analyses. Approximations of single exponential fits were performed after normalizing the response to the maximal amplitude and fitting the last 20% of the tail current.

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References

Ashburner, M. (1989). Drosophila: A Laboratory Handbook. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Baer, A. M., and Saibil, H. R. (1988). Light- and GTP-activated hydrolysis of phosphatidylinositol bisphosphate in squid photoreceptor membranes. J. Biol. Chem. *263*, 17–20.

Berridge, M. J., and Irvine, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature *312*, 315–321.

Berridge, M. J., and Irvine, R. F. (1989). Inositol phosphates and cell signalling. Nature *341*, 197–205.

Birchmeier, C., O'Neill, K., Riggs, M., and Wigler, M. (1990). Characterization of ROS1 cDNA from a human glioblastoma cell line. Proc. Natl. Acad. Sci. USA *87*, 4799–4803.

Bloomquist, B., Shortridge, R., Schneuwly, S., Perdew, M., Montell, C., Steller, H., Rubin, G., and Pak, W. (1988). Isolation of a putative phospholipase C gene, *norpA*, and its role in phototransduction. Cell *54*, 723–733.

Cavener, D. R. (1987). Comparison of the consensus sequence flanking translational start site in Drosophila and vertebrates. Nucleic Acids Res. *15*, 1353–1361.

Cho, K.-O., Hunt, C. A., and Kennedy, M. B. (1992). The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron *9*, 929–942.

Cowman, A. F., Zuker, C. S., and Rubin, G. R. (1986). An opsin gene expressed in only one photoreceptor cell type of the Drosophila eye. Cell *44*, 705–710.

Devary, O., Heichal, O., Blumenfeld, A., Cassel, D., Suss, E., Barash, S., Rubinstein, C. T., Minke, B., and Selinger, Z. (1987). Coupling of photoexcited rhodopsin to inositol phospholipid hydrolysis in fly photoreceptors. Proc. Natl. Acad. Sci. USA *84*, 6939–6943.

Fein, A. (1986). Excitation and adaptation of *Limulus* photoreceptors by light and inositol 1,4,5-trisphosphate. Trends Neurosci. *93*, 110–114.

Ferris, C. D., and Snyder, S. H. (1992). Inositol phosphate receptors

and calcium disposition in the brain. J. Neurosci. *12*, 1567–1574.

Hafen, E., Basler, K., Edstrom, J. E., and Rubin, G. M. (1987). *sevenless*, a cell-specific homeotic gene of Drosophila, encodes a putative transmembrane receptor with a tyrosine kinase domain. Science *236*, 55–63.

Hardie, R. C., and Minke, B. (1992). The *trp* gene is essential for a light-activated Ca^{2+} channel in Drosophila photoreceptors. Neuron *8*, 643–651.

Hardie, R. C., Voss, D., Pongs, L., and Laughlin, S. B. (1991). Novel potassium channels encoded by the *Shaker* gene in Drosophila photoreceptors. Neuron *6*, 477–486.

Hardie, R. C., Peretz, A., Suss-Toby, E., Rom-Glas, A., Bishop, S. A., Selinger, Z., and Minke, B. (1993). Protein kinase C is required for light adaptation in *Drosophila* photoreceptors. Nature *363*, 634–637.

Harlow, E., and Lane, D. (1988). Antibodies: A Laboratory Manual. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Henzi, V., and MacDermott, A. B. (1992). Characterization and function of Ca^{2+}- and inositol 1,4,5-trisphosphate-releasable stores of Ca^{2+} in neurons. Neuroscience *46*, 251–273.

Hurley, J. B. (1992). Signal transduction enzymes of vertebrate photoreceptors. J. Bioenerg. Biomembr. *24*, 219–226.

Ito, M., Nagafuchi, A., Yonemura, S., Kitani-Yasuda, T., Tsukita, S., and Tsukita, S. (1993). The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J. Cell Biol. *121*, 491–502.

Johnson, E. C., Robinson, P. R., and Lisman, J. E. (1986). Cyclic GMP is involved in the excitation of invertebrate photoreceptors. Nature *324*, 468–470.

Kennelly, P. J., and Krebs, E. G. (1991). Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J. Biol. Chem. *266*, 15555–15558.

Lagnado, L., and Baylor, D. (1992). Signal flow in visual transduction. Neuron *8*, 995–1002.

Lee, Y.-J., Dobbs, M. B., Verardi, M. L., and Hyde, D. R. (1990). *dgq*: a Drosophila gene encoding a visual system–specific Gα molecule. Neuron *5*, 889–898.

Lindsley, D., and Zimm, G. (1992). The Genome of *Drosophila melanogaster*. (San Diego: Academic Press).

Lisman, J. E., and Brown, J. E. (1972). The effects of intracellular iontophoretic injection of calcium and sodium ions on the light response of *Limulus* ventral photoreceptors. J. Gen. Physiol. *59*, 701–719.

Montell, C., Jones, K., Zuker, C. S., and Rubin, G. R. (1987). A second opsin gene expressed in the ultraviolet-sensitive R7 photoreceptor cells of *Drosophila melanogaster*. J. Neurosci. *7*, 1558–1566.

Nishizuka, Y. (1986). Studies and perspectives of protein kinase C. Science *223*, 305–312.

O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, J., Pak, W. L., and Applebury, M. L. (1985). The Drosophila *ninaE* gene encodes an opsin. Cell *40*, 839–850.

Pak, W. L. (1979). Study of photoreceptor function using Drosophila mutants. In Neurogenetics: Genetic Approaches to the Nervous System, X. O. Breakfield, ed. (New York: Elsevier), pp. 67–99.

Ranganathan, R., Harris, G. L., Stevens, C. F., and Zuker, C. S. (1991). A *Drosophila* mutant defective in extracellular calcium-dependent photoreceptor inactivation and adaptation. Nature *354*, 230–232.

Rosenberg, A. H., Lade, B. N., Chui, D., Lin, S., Dunn, J. J., and Studier, F. W. (1987). Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene *56*, 125.

Rubin, G. M., and Spradling, A. C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. Science *218*, 348–353.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Shieh, B.-H., Stamnes, M., Seavello, S., Harris, G., and Zuker, C. (1989). The *ninaA* gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein. Nature *338*, 67–70.

Smith, D. P., Shieh, B.-H., and Zuker, C. S. (1990). Isolation and structure of an arrestin gene from Drosophila. Proc. Natl. Acad. Sci. USA *87*, 1003–1007.

Smith, D. P., Ranganathan, R., Hardy, R. W., Marx, J., Tsuchida, T., and Zuker, C. S. (1991). Photoreceptor deactivation and retinal degeneration mediated by a photoreceptor-specific protein kinase C. Science *254*, 1478–1484.

Sonnenberg, E., Godecke, A., Walter, B., Bladt, F., and Birchmeier, C. (1991). Transient and locally restricted expression of the ros1 protooncogene during mouse development. EMBO J. *10*, 3693–3702.

Stryer, L. (1986). Cyclic GMP cascade of vision. Annu. Rev. Neurosci. *9*, 87–119.

Studier, F. W., and Moffatt, B. A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned gene. J. Mol. Biol. *189*, 113–130.

Sved, J. (1986). Technical note. Drosophila Info. Serv. *73*, 169.

Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T., and Nishizuka, Y. (1979). Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J. Biol. Chem. *254*, 3692–3695.

Tessarollo, L., Nagarajan, L., and Parada, L. F. (1992). c-ros: the vertebrate homolog of the sevenless tyrosine kinase receptor is tightly regulated during organogenesis in mouse embryonic development. Development *115*, 11–20.

Thomas, G. M., Cunningham, E., Fensome, A., Ball, A., Totty, N. F., Truong, O., Hsuan, J. J., and Cockcroft, S. (1993). An essential role for phosphatidylinositol transfer protein in phospholipase C-mediated inositol lipid signaling. Cell *74*, 919–928.

Woods, D. F., and Bryant, P. J. (1991). The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell *66*, 451–464.

Yarfitz, S., Niemi, G. A., McConnell, J. L., Fitch, C. L., and Hurley, J. B. (1991). A Gβ protein in the Drosophila compound eye is different from that in the brain. Neuron *7*, 429–438.

Yoshioka, T., Inoue, H., and Hotta, Y. (1984). Absence of phosphatidylinositol phosphodiesterase in the head of a Drosophila visual mutant, *norpA*. Biochem. J. *100*, 1015–1022.

Zuker, C. S., Cowman, A. F., and Rubin, G. M. (1985). Isolation and structure of a rhodopsin gene from Drosophila. Cell *40*, 851–858.

Zuker, C. S., Montell, C., Jones, K., Laverty, T., and Rubin, G. R. (1987). A rhodopsin gene expressed in photoreceptor cell R7 of the Drosophila eye: homologies with other signal-transducing molecules. J. Neurosci. *7*, 1550–1557.

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